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Abstract

The effect of ionizing radiation on jejunal mucosal blood flow was investigated in rats receiving whole body exposure to 10 Gy γ-radiation. Irradiation resulted in increased myeloperoxidase activity, indicative of neutrophil infiltration, and prostaglandin E_2 synthesis by 2 hr post-irradiation, but had no effect on leukotriene B_4 synthesis. In *ex vivo* intestinal chamber studies, exposure to radiation reversed the mucosal blood flow changes elicited by intravenous administration of leukotriene C_4 (1 µg/kg/min for 10 min) or endothelin-1 (1 µg/kg/min for 5 min), but not those elicited by topical capsaicin (100 or 640 µM) as measured by laser Doppler flowmetry. The effect of radiation on vascular responses to leukotriene C_4, but not those to endothelin-1, was reversed by pretreatment of the rats with the cyclo-oxygenase inhibitor, indomethacin (5 mg/kg). There was no significant effect of irradiation on mean arterial pressure or on ion or protein effluxes into the chamber bathing solution, nor did irradiation alter transmural potential difference. These studies point to altered responsiveness of the jejunal mucosal vasculature shortly after exposure to ionizing radiation. Changes in function could reflect the onset of an acute inflammatory response, and appear to have cyclo-oxygenase-dependent and independent components.

Résumé

Les effets de la radiation ionisante sur le débit sanguin de la muqueuse jéjunale ont été étudiés chez le rat ayant été exposé à 10 Gy de radiation gamma. L'irradiation a causé une augmentation de l'activité de la myélopéroxydase, ce qui reflète une infiltration de neutrophiles, et aussi une augmentation de la synthèse de la prostaglandine E_2 deux heures post-irradiation, mais n'a eu aucun effet sur la synthèse de la leucotriene B_4. Des études *ex-vivo* ont démontré que, suite à l'administration intraveineuse de leucotriene C_4 (1 µg/kg/min pour 10 min) ou d'endothéline-1 (1 µg/kg/min pour 5 min), l'exposition à la radiation a inversé les changements observés dans le débit sanguin tel que mesuré par l'analyse Doppler au laser. Ce phénomène n'a pas été observé suite à l'administration topique de capsaicin (100 ou 640 µM). L'effet de la radiation sur la réaction vasculaire causée par la leucotriene C_4 , et non par l'endothéline-1, a été inversé suite à un pré-traitement des rats avec un inhibiteur de la cyclo-oxygénase, l'indométhacin (5 mg/kg). L'irradiation n'a eu aucun effet significatif sur la tension artérielle moyenne, ou sur le flux d'ions ou de protéines dans les solutions des chambres intestinales; de plus, elle n'a pas modifié la différence de potentiel transmural. Ces études démontrent une altération de la vascularisation de la muqueuse jéjunale peu après l'exposition à la radiation ionisante. Ces changements fonctionnels pourraient refléter le début d'une réaction inflammatoire aiguë, et semblent

être associés à des éléments reliés ou indépendants de la cyclo-oxygénase.

EXECUTIVE SUMMARY

Canadian Forces personnel may find themselves operating in environments of moderate to high doses of ionizing radiation. While the life-threatening and performance-degrading effects of radiation are known, the mechanisms which underlie the manifestation of symptoms are unclear. This has hampered the discovery and development of effective prophylactic or therapeutic agents.

The maintenance of adequate blood flow through the intestine is required for preservation of mucosal structure and function. The pathophysiological gastrointestinal effects of ionizing radiation may be due in part to altered blood flow responses post-exposure. The present study was designed to investigate the effects of ionizing radiation on the vascular response to various vasoactive mediators, including leukotriene C_4, endothelin-1 and capsaicin. Radiation reversed the vasoconstrictive effects of leukotriene C_4 in a prostaglandin-dependent manner. Radiation also reversed the initial vasodilatory response to endothelin-1, but in a prostaglandin-independent manner. The vasodilatory response to sensory afferent neural stimulation by capsaicin was unchanged. Other aspects of mucosal function, including ion fluxes, protein flux and the development of transmural potential difference, were not affected by radiation exposure.

The data indicate that there are multiple alterations in the response of the intestinal vasculature to vasoactive mediators post-irradiation. These may correlate with the onset of an acute inflammatory response, as demonstrated by increases in myeloperoxidase activity and prostaglandin E_2 synthesis.

These studies increase our understanding of intestinal pathophysiology post-irradiation, and may ultimately contribute to the identification and development of suitable therapeutic intervention in the manifestation of gastrointestinal prodromal symptoms of exposure.

1.0 INTRODUCTION

The acute radiation syndrome which occurs following exposure to ionizing radiation of over 100 cGy consists of various subsyndromes, the manifestation of which is dependent upon the dose received. All subsyndromes, however, display prodromal symptoms such as nausea and vomiting, diarrhea, and fatigue, all of which represent a potentially significant performance decrement. Exposure to radiation may also lead to the development of chronic inflammatory responses and associated pathology.

While most studies have investigated the longer term (weeks to months) biological effects of ionizing radiation, some studies indicate acute effects within the first few hours following exposure. For example, Buell and Harding (2) noted a significant increase in both neutrophil influx and tissue hemoglobin by 4 hr post-irradiation. Gunter-Smith (4) observed altered electrolyte transport in rabbit ileum by 24 hr post-irradiation. Recently (8), we described effects of irradiation on rat ileal electrolyte transport, and suggested that these could be related to alterations in mast cell-nerve interactions. Other experimental studies have shown significant alterations in mucosal blood flow (5,10,15). Maintenance of adequate mucosal blood flow is essential for preservation of mucosal integrity, with impairment of flow predisposing the gastrointestinal tract to injury.

The mechanism underlying these effects is unclear. Elevated concentrations of inflammatory mediators in the mucosa or submucosa may significantly affect vasoreactivity, and may contribute to radiation-induced intestinal pathophysiology. Identification of the exact nature of these responses is necessary before therapeutic or prophylactic modalities can be applied. Therefore, we have investigated the effects of ionizing radiation on the control of blood flow *ex vivo*. Particular emphasis has been placed on responses to various mediators known to play an integral role in controlling mucosal blood flow.

2.0 METHODS

2.1 ANIMALS

Male Sprague-Dawley rats (225-275 g; Charles River) were housed in plastic holding cages under controlled temperature (22°C) and photoperiod (12 h light-dark cycle). They were fed standard rat chow and water *ad libitum*. Rats were acclimatized to these conditions for at least 5 days prior to use. All procedures were carried out in accordance with the guidelines established by the Canadian Council on Animal Care.

Rats were exposed to a 10 Gy whole body dose of γ-radiation from a ^{137}Cs source (GammaCell 40 Irradiator, Nordion, Kanata, ON) at a dose rate of 1.2 Gy/min. A group of "sham-irradiated" animals was placed within the apparatus but was not exposed to the source.

2.2 BIOCHEMICAL ANALYSES

Segments of jejunum were taken in order to determine radiation-induced changes in tissue levels of various mediators of inflammation in rats killed at 0.5, 2, 24 and 48 hr post-irradiation. Myeloperoxidase (MPO) activity, an indicator of neutrophil infiltration, was measured spectrophotometrically using a modification of the method previously described by Krawisz *et al.* (6). Samples were removed, weighed and immediately frozen with liquid nitrogen. They were stored at -70°C for subsequent measurement of MPO activity. Tissue levels of 5-HT were measured using the method of Curzon and Green (3). Histamine was measured using the radioenzymatic assay of Shaff and Beavan (14). Determination of the synthetic capacity of the tissue for PGE_2 and LTB_4 was carried out as previously described (19) using commercially available radioimmunoassay kits (PGE_2, Amersham; LTB_4, NEN-Dupont).

2.3 EX VIVO INTESTINAL CHAMBER PREPARATION

The onset of acute inflammation, as indicated by MPO and PGE_2 data (see results), occurred by 2 hr post-irradiation. Thus, subsequent experiments were conducted in animals after either sham treatment or at 2 hr post-irradiation. Rats were anesthetized with sodium pentobarbital (Somnotol, 60 mg/kg i.p.). A jugular vein was cannulated for the adminstration of drugs and a carotid artery was cannulated for the measurement of mean arterial pressure. A laparotomy was performed and an 8 cm segment of jejunum exposed. The segment was pulled through an aperture in a lucite plate, opened along the antimesenteric border and pinned mucosal side up. A plexiglass chamber was clamped to the intestinal surface such that a reservoir was formed with the mucosa comprising the bottom. This preparation is similar to that initially developed for use in

the stomach (12). Care was taken to not disrupt blood and nerve supplies. The mucosal surface was gently rinsed with warm (37°C) saline. Experiments were conducted with 8 sequential 5-min periods during which the mucosa was bathed with 3 ml isotonic mannitol (pH 7.0, 37°C). This bathing solution was changed at the end of each 5-min period and saved for determination of fluxes of K^+ and Na^+ by flame photometer (Corning) and for Cl^- with a chloride analyzer (Corning). Efflux of protein into the bathing solution was measured by means of a protein-dye binding assay (BioRad). Potential difference (PD) was measured across the tissue by means of calomel electrodes connected via agar/3 M KCl bridges placed in the bathing solution and in the peritoneum. A laser-Doppler probe (TSI LaserFlo) was lowered using a micromanipulator onto the surface of the mucosa for the continuous measurement of mucosal/submucosal blood flow. Mucosal blood flow and mean arterial pressure were recorded and subsequently analyzed using a computerized data acquisition system (MacLab).

At the beginning of period 5, rats were infused intravenously with either leukotriene (LT) C_4 (0.3, 1.0 or 3.0 µg/kg/min for 10 min) or endothelin (ET)-1 (0.3 or 1.0 µg/kg/min) for 5 min. These doses and infusion times were chosen based on those known to have vasoactive effects in a similar *ex vivo* gastric preparation (7,18). Other experiments were designed to investigate the effect of radiation on capsaicin-induced activation of primary sensory afferent neurons in the rat jejunum. Stimulation of sensory afferent neurons by capsaicin causes both dilation of submucosal arterioles (1,11) and chloride secretion (9) in the guinea pig ileum *in vitro*. Capsaicin (100 or 640 µM) was added to the bathing solution at the beginning of period 5. After 5 min this solution was replaced with isotonic mannitol.

Experiments with 1.0 µg/kg/min LTC_4, 1.0 µg/kg/min ET-1 and 100 µM capsaicin were repeated in rats pretreated with the cyclo-oxygenase inhibitor, indomethacin (5 mg/kg, s.c.) 60 min prior to drug administration.

2.4 HISTOLOGICAL DETECTION OF MAST CELLS

Mucosal mast cell degranulation is associated with increased gastrointestinal blood flow (17). In addition, we have shown that exposure of rats to 10 Gy γ-radiation causes a reduction in ileal mucosal mast cell number by 24 hr (8). Thus, radiation-induced mast cell depletion was determined in jejunal samples in a separate series of experiments. Rats were irradiated as described above and killed at 2, 24 and 48 h post-irradiation. Controls were sham-treated. Full-thickness segments of ileum were pinned onto wax dishes and were fixed in Carnoy's fixative for 2 hr. They were stored in 70% ethanol and were subsequently embedded in paraffin for sectioning. Sections (6 µm thick) were mounted on glass slides, re-hydrated and stained with 1.0% astra blue/0.5% safranin O for the detection of mast cells, or were post-fixed with picric

acid and stained with hematoxylin/phloxine-B/saffran for routine micrography on a Zeiss Axiophot photomicroscope using Ilford PanF 135 film. Mast cells in the jejunal mucosa were counted and expressed per crypt-villus unit. Villus height and crypt depth were measured using an ocular micrometer.

2.5 DRUGS AND REAGENTS

Stock solutions of LTC_4 (Cayman Chemical, Ann Arbor, Michigan) and of ET-1 (Bachem California, Torrance, CA) were dissolved in distilled water, aliquoted and frozen at -70°C. Aliquots were thawed immediately prior to use. Capsaicin (Sigma Chemical Co., St. Louis, Missouri) was dissolved in Tween 80:ethanol:saline (4:1:1) and stored at 4°C. Indomethacin was dissolved in 1.25% sodium bicarbonate immediately prior to use. Other chemicals were purchased from either Sigma or BDH (Toronto, Ontario).

2.6 STATISTICS

Data are expressed as the mean ± SEM of at least 5 animals in each group. Comparisons of experimental groups to sham-treated groups were conducted with Student's t-test for unpaired data. In all cases, an associated probability (p) value of less than or equal to 0.05 was considered to be significant.

3.0 RESULTS

3.1 BIOCHEMICAL ANALYSES

MPO activity was measured as the indicator of the onset of the inflammatory response. MPO activity was elevated above sham levels in tissue taken from rats killed 2 hr post-irradiation, indicating a rapid, radiation-induced influx of neutrophils into the intestinal mucosa. There were increases of 72% and 90% at 2 and 24 hrs post-irradiation respectively (Figure 1). Pretreatment of sham-treated rats with indomethacin did not significantly alter MPO activity (data not shown). There was a significant, 3-fold increase in PGE_2 synthesis in tissue taken 2 hr post-irradiation (sham, 28.0 ± 7.5 pg/mg tissue; 2 hr post-irradiation, 84.1 ± 17.8 pg/mg tissue; Figure 2). There were no significant differences at other time points. There were no radiation-induced differences in LTB_4 synthesis compared to shams (Figure 2). The tissue content of 5-HT was significantly lower in tissues taken 2 hr post-irradiation (32% lower than shams; Figure 3). The content of 5-HT in tissues taken at other time points post-irradiation was not significantly different from the sham-treated group. Tissue levels of histamine were significantly reduced compared to shams at 24 hr post-irradiation (90% reduction; $p<0.001$), but were unchanged at 0.5 and 2 hr (Figure 3).

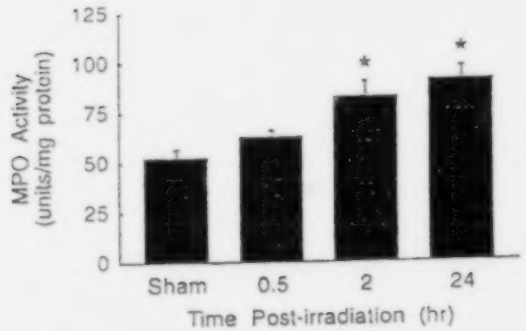

Figure 1. Myeloperoxidase (MPO) activity was measured in samples of jejunum taken from sham-treated rats and from rats killed at 0.5, 2 and 24 hrs following exposure to 10 Gy ionizing radiation. *$p<0.05$ vs sham.

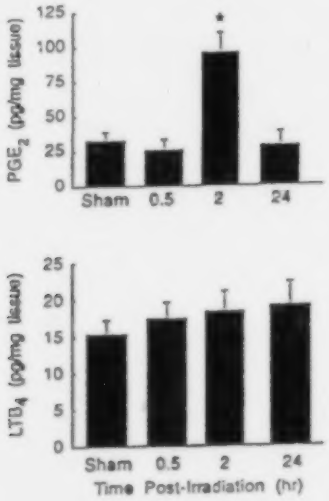

Figure 2. *Top panel.* Prostaglandin E_2 synthesis. There was a significant increase in PGE_2 synthesis at 2 hr post-irradiation compared to shams. *p<0.05 vs sham. *Bottom panel.* There was no change in jejunal leukotriene B_4 synthesis at any time post-irradiation compared to shams.

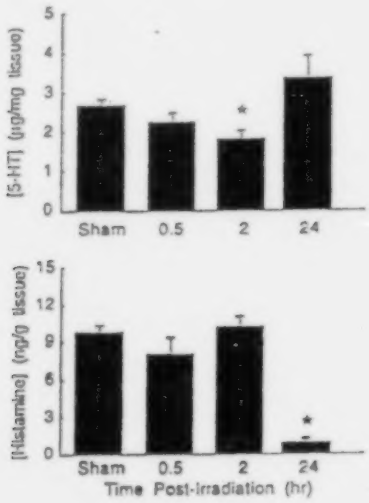

Figure 3. *Top panel.* Tissue content of 5-hydroxytryptamine was significantly depressed in samples of jejunum taken from rats killed at 2 hr post-irradiation. *p<0.05 vs sham. *Bottom panel.* Tissue content of histamine was significantly lower in jejunum taken from rats killed at 24 hr post-irradiation. *p<0.01 vs sham.

3.2 EX VIVO INTESTINAL CHAMBER PREPARATION

Because MPO activity and PGE_2 synthesis were elevated at 2 hr post-irradiation, subsequent studies on the effects of ionizing radiation on jejunal mucosal function were conducted at this time point post-irradiation.

In *ex vivo* intestinal chamber studies, PD and ion and protein effluxes were measured as indices of epithelial integrity. There were no differences in K^+, Na^+ or Cl^- efflux into the bathing solution when irradiated rats were compared to shams. Intravenous infusion of either LTC_4 or ET-1 had no effect on ion effluxes in either group. Similarly, topical exposure to capsaicin did not affect ion effluxes. In pre-infusion periods (periods 1-4), protein efflux was not significantly different between shams and irradiated rats. However, following infusion of 1 µg/kg/min LTC_4 there was a significantly lower protein efflux in the irradiated rats compared to shams (Figure 4). PD was significantly higher in periods 9 and 10 in irradiated rats receiving LTC_4 (Figure 4). These differences were not apparent in groups receiving indomethacin pretreatment. There were no significant differences in PD or protein efflux between irradiated and sham rats receiving 1 µg/kg/min ET-1 (Figure 5). Topical exposure of the jejunum to capsaicin did not significantly affect PD, but caused a large (~20-fold) increase in protein efflux during the time it was in contact with the mucosa. Exposure to radiation did not significantly alter this effect (Figure 6).

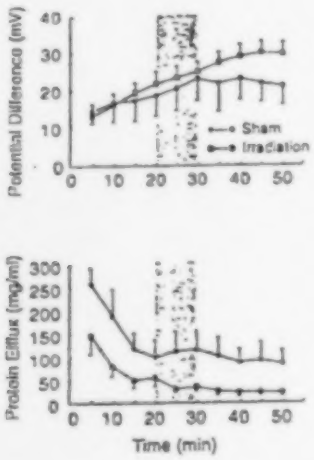

Figure 4. Intravenous infusion of 1 µg/kg/min of leukotriene C_4 for 10 min. The shaded bar indicates period of infusion. *Top panel.* Transmural potential difference across jejunum in *ex vivo* chamber. *Bottom panel.* Protein concentration in bathing solutions in *ex vivo* chamber at the end of each period. Solutions were removed and replaced with fresh isotonic mannitol at the end of each 5 minute period.

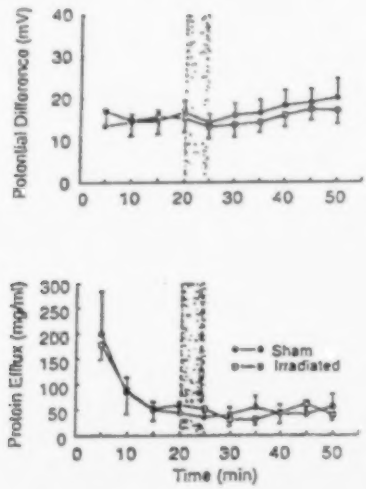

Figure 5. Intravenous infusion of 1 µg/kg/min of endothelin-1 for 5 min. The shaded bar indicates the period of infusion. *Top panel.* Transmural potential difference across the jejunum in *ex vivo* chamber. *Bottom panel.* Protein concentration in bathing solutions in *ex vivo* chamber at the end of each period. Solutions were removed and replaced with fresh isotonic mannitol at the end of each 5 minute period.

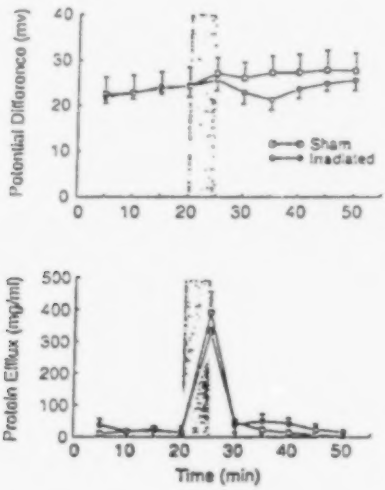

Figure 6. Topical administration of 100 µM capsaicin for 5 min. The shaded bar indicates the period during which capsaicin was in contact with the mucosal surface. *Top panel.* Transmural potential difference across the jejunum in *ex vivo* chamber. *Bottom panel.* Protein concentration in bathing solutions in *ex vivo* chamber at the end of each period. Solutions were removed and replaced with fresh isotonic mannitol at the end of each 5 minute period. Protein concentration increased significantly in both sham and irradiated animals during the time capsaicin was in contact with the mucosa ($p<0.05$ compared to all other groups; ANOVA with *post hoc* Newman-Keuls test).

Basal blood flow varied depending upon the region of jejunum in contact with the laser-Doppler probe. Hence, it was not possible to reliably determine differences in basal mucosal blood flow measurements between irradiated and sham rats. Therefore, for each experiment a region was chosen which had a basal flow of approximately 120 ml/100 g tissue/min in order to have a consistent starting point. Intravenous infusion of 1 or 3 µg/kg/min LTC_4 caused a significant decrease in blood flow (Figure 7), where as 0.3 µg/kg/min was without affect. In irradiated rats, infusion of 1 µg/kg/min LTC_4 resulted in an increase in flow (14.5 ± 3.6%; $p<0.001$ vs sham). Pretreatment with indomethacin eliminated this difference (sham, -6.4 ± 17.8%; irradiated, -12.2 ± 24.1%; $p>0.05$) although there was substantial variation in the responses to LTC_4 in each indomethacin group (Figure 8). Infusion of 3 µg/kg/min LTC_4 to irradiated rats resulted in a decrease in blood flow which was not different from that observed in shams receiving this dose. Infusion of LTC_4 did not alter mean arterial pressure in any of the treatment groups.

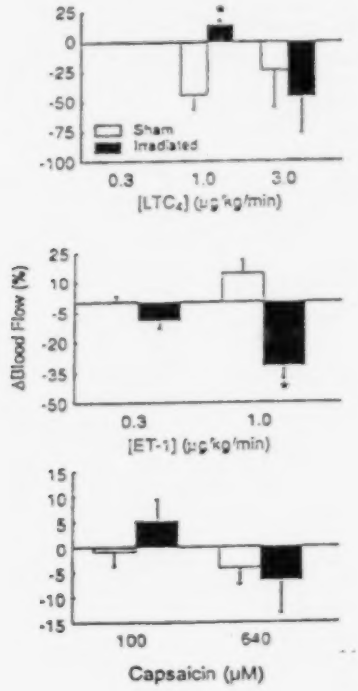

Figure 7. Change in blood flow as a result of administration of 0.3, 1.0 or 3.0 µg/kg/min leukotriene C_4 i.v. for 10 min (*top panel*), 0.3 or 1.0 µg/kg/min endothelin-1 i.v. for 5 min (*middle panel*), or 100 or 640 µM capsaicin topically for 5 min (*bottom panel*). Open bars represent sham treated rats, whereas solid bars represent rats exposed to 10 Gy γ-radiation. Blood flow is expressed as the percent change from baseline as measured with a laser Doppler flowmeter. *$p<0.05$ vs sham.

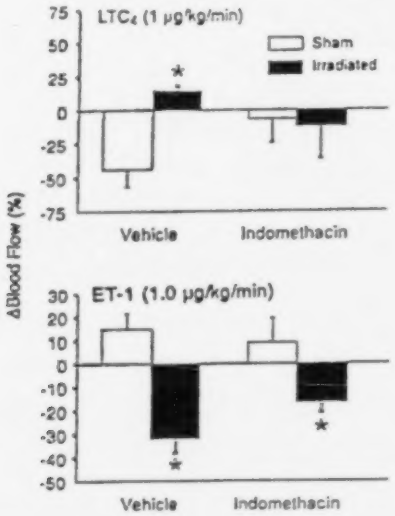

Figure 8. Effect of subcutaneous adminstration of 5 mg/kg indomethacin 60 min prior to intravenous infusion of either leukotriene C_4 (1 µg/kg/min for 10 min) or endothelin-1 (1 µg/kg/min for 5 min) on blood flow in jejunum mounted in *ex vivo* chamber. Open bars represent sham treated rat, whereas solid bars represent rats exposed to 10 Gy γ-radiation. Blood flow is expressed as the percent change from baseline as measured with a laser Doppler flowmeter. *$p<0.05$ vs sham.

ET-1 infusion at either 0.3 or 1 µg/kg/min for 5 min caused a characteristic increase in flow in sham treated rats (Figure 7), followed by a decrease in flow. These observations were reflected in changes in mean arterial pressure, where there was an initial transient drop in blood pressure, followed by a sustained increase as has been previously described in the rat (16). In irradiated rats, 1 µg/kg/min ET-1 infusion resulted in a significant decrease in flow (-31.8 ± 6.3% below baseline; $p<0.001$ vs sham). This trend was not affected by indomethacin pretreatment (Figure 8).

Topical capsaicin at 100 or 640 µM caused a slight reduction in blood flow in shams. This was not significantly affected by prior exposure to radiation (Figure 7).

Hematocrit tended to increase by approximately 5-6% over the course of the experiments. There were, however, no significant differences among any of the groups (data not shown).

3.3 HISTOLOGY

The number of mucosal mast cells which could be detected with the astra blue-safranin stain did not differ from shams at 2 hr post-irradiation. By 24 hr, however, mast cells were virtually undetectable (Table 1). Villus height and crypt depth were unchanged compared to controls.

Table 1. Mast cell counts and villus-crypt length in rat jejunum post-irradiation.

Time Post-Irradiation	Mast cells per villus-crypt unit	Villus height (mm)	Crypt depth (mm)
Sham	8.9 ± 0.9	0.42 ± 0.01	0.10 ± 0.01
2 hr	7.9 ± 0.8	0.44 ± 0.01	0.09 ± 0.004
24 hr	0.2 ± 0.01**	0.42 ± 0.01	0.08 ± 0.01

**$p<0.01$ vs. sham

4.0 DISCUSSION

CF personnel may be called upon to perform their duties in radiation environments. Exposure to ionizing radiation, such as that which might be received during conduct of these duties, is most commonly associated with nausea, vomiting and diarrhea. Other side effects develop more slowly, and include the development of chronic intestinal inflammation and morphological damage, often weeks to months post-treatment. However, acute inflammatory responses have been observed post-irradiation, following a time course that parallels the development of these prodromal symptoms. Histologically detectable neutrophil influx and evidence of microvascular damage occur in all regions of the gastrointestinal tract within the first 4 hours after exposure to 10 Gy radiation in rats (2). We have investigated the possibility that this inflammatory response may alter normal jejunal mucosal function in the rat.

Exposure to ionizing radiation resulted in a modest increase in MPO activity, confirming previous reports of neutrophil influx (2). However, the increase was not large compared to other models of intestinal inflammation, such as that which occurs following intraluminal administration of trinitrobenzenesulfonic acid to guinea pig ileum (13). Furthermore, there was no increase in LTB_4 synthesis at any time post-irradiation. PGE_2 synthesis increased transiently at 2 hr post-irradiation. Excised jejunal segments appeared macroscopically normal, and exhibited few signs of damage histologically, precluding structural changes in the observed physiological alterations. Similar histological findings have recently been reported in rat (8) and rabbit (4) ileum.

Despite the overt lack of tissue damage, several aspects of mucosal function were altered post-irradiation. First, vasoreactivity to LTC_4 and ET-1 was reversed 2 hr post-irradiation. In sham treated animals, intravenous LTC_4 induced a characteristic decrease in flow as measured by laser-Doppler velocimetry, indicative of vasoconstriction. We (18) and others have reported on the potent vasoconstrictive effects of the peptidleukotrienes in the gastrointestinal tract. In contrast, intravenous administration of LTC_4 to irradiated rats resulted in a vasodilatory response, as indicated by an increase in blood flow. This effect could have been due either to a decrease in the ability

of the vasculature to constrict post-irradiation, or to the production of an endogenous vasodilator post-irradiation. The first possibility is precluded by studies with 3 µg/kg/min LTC_4, which induced a large decrease in blood flow in both shams and irradiated rats. Studies with indomethacin pretreatment implicate an endogenous prostanoid in the dilatory response to LTC_4, since cyclo-oxygenase blockade resulted in a LTC_4-induced decrease in flow in irradiated rats. In corroboration of the present study, others have reported that responsiveness of rat abdominal aorta *in vitro* to the thromboxane mimetic, U46619, a potent vasoconstrictor, was significantly depressed post-irradiation. This effect was reversed by indomethacin pretreatment of the tissue, again implicating the involvement of an endogenous prostanoid (20).

ET-1, considered a potent vasoconstrictor, had a biphasic effect in our *ex vivo* intestinal chamber, causing an initial increase in flow, followed by a prolonged decrease in flow. This pattern is typical of i.v. administration of ET in the rat (16). Irradiation inhibited the dilatory phase of the ET-1 response, an effect which was not reversed by indomethacin pretreatment. Relaxation of rabbit ear artery is attenuated following exposure to X-irradiation as well (10). It is apparent, therefore, that radiation causes different effects on the vasculature, which have cyclo-oxygenase dependent and independent mechanisms. Some evidence exists which implicates nitric oxide in the dilatory response to ET-1. We have not investigated the effects of radiation on nitric oxide synthase activity in the present study.

These studies provide evidence that exposure to ionizing radiation can significantly alter normal physiological responses in the rat jejunum. Changes in vasoreactivity appear to be multifactorial, having both cyclo-oxygenase dependent and independent components. The results with endothelin are intriguing, given its potency and physiological relevance. Further studies will expand on the potential role of radiation on nitric oxide synthase activity in relation to altered mucosal vasoreactivity. It may be in this area that a link may be made between radiation-induced changes in the expression of endogenous mediators, and the manifestation of the gastrointestinal prodromal symptoms (ie. diarrhea) of exposure to ionizing radiation.

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The effect of ionizing radiation on jejunal mucosal blood flow was investigated in rats receiving whole body exposure to 10 Gy γ-radiation. Irradiation resulted in increased myeloperoxidase activity, indicative of neutrophil infiltration, and prostaglandin E_2 synthesis by 2 hr post-irradiation, but had no effect on leukotriene B_4 synthesis. In *ex vivo* intestinal chamber studies, exposure to radiation reversed the mucosal blood flow changes elicited by intravenous administration of leukotriene C_4 (1 µg/kg/min for 10 min) or endothelin-1 (1 µg/kg/min for 5 min), but not those elicited by topical capsaicin (100 or 640 µM) as measured by laser Doppler flowmetry. The effect of radiation on vascular responses to leukotriene C_4, but not those to endothelin-1, was reversed by pretreatment of the rats with the cyclo-oxygenase inhibitor, indomethacin (5 mg/kg). There was no significant effect of irradiation on mean arterial pressure or on ion or protein effluxes into the chamber bathing solution, nor did irradiation alter transmural potential difference. These studies point to altered responsiveness of the jejunal mucosal vasculature shortly after exposure to ionizing radiation. Changes in function could reflect the onset of an acute inflammatory response, and appear to have cyclo-oxygenase-dependent and independent components.

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